



Effect of Commercial Bread Improvers on Microbial Quality, Storage Stability, and Freshness Characteristics of Wheat–Cassava Composite Bread

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Abstract

Composite flour made from Wheat-cassava bread offers a convenient method using locally available cassava flour, but incorporating wheat substitution can alter dough structure and accelerate quality deterioration during storage. This work study the effects of four commercial bread improvers such as guar gum, ascorbic acid, Panok® and STK Royal, on the microbial quality and freshness characteristics of bread prepared from a 90:10 wheat-cassava composite flour. Each improver was incorporated at 0.5, 1.0, 2.0 and 3.0% (w/w, based on composite flour), with bread produced without the test improver serving as the control. Total viable bacterial, coliform and mould counts were determined by culture-based methods, while storage stability was assessed from crumb moisture and a calculated freshness index during four days of ambient storage. Ascorbic-acid breads showed the lowest bacterial counts (2.47×10^2 to 2.87×10^2 CFU/g). Guar-gum breads showed the lowest mould counts (2.37×10^2 to 2.67×10^2 CFU/g), whereas the control had a mould count of 5.83×10^3 CFU/g. No coliforms were detected in the fresh bread samples. Freshness declined during storage in all treatments. On day 4, STK Royal bread retained the greatest crumb moisture (11.75%) and freshness index (73.00 %), whereas the control had the lowest values (8.51% and 60.75%). Presumptive isolates belonged to *Bacillus*, *Micrococcus*, *Staphylococcus*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor*. The findings show that the improvers did not provide a uniform response: ascorbic acid was associated with the lowest bacterial load, guar gum with the lowest mould load, and STK Royal with the strongest moisture and freshness retention. These treatment-specific effects were considered separately while formulating wheat-cassava composite bread.

Keywords: Bread improvers, microbial quality, mould, storage stability, staling, freshness

1.0 Introduction

Introduction

Bread is a widely consumed staple food valued for convenience, palatability and versatility. In countries that depend heavily on imported wheat, partial replacement of wheat flour with locally produced root and tuber flours can support raw-material diversification and reduce pressure on wheat supply. Cassava is relevant in tropical food systems because it is widely cultivated and can be processed into flour suitable for composite bakery formulations. Recent work has confirmed that increasing cassava substitution changes the functional and dough properties of wheat-cassava flour systems, principally through dilution of gluten-forming proteins and changes in water

absorption and pasting behaviour leading to structural collapse (Li et al., 2023). Although composite flour creates economic and food-system opportunities, reduced gluten continuity can retard gas retention, loaf structure and crumb characteristics. Bread quality also changes after baking through moisture redistribution and starch retrogradation. These processes contribute to crumb firming and loss of freshness during storage. Contemporary bread studies therefore distinguish between physical staling and microbiological deterioration rather than treating shelf life as a single outcome (Chang et al., 2023; Pyo et al., 2024).

Bread improvers are used to modify dough handling, gas retention, crumb structure and storage behaviour. Hydrocolloids can increase water binding and alter starch-protein interactions, thereby influencing crumb softness and staling kinetics. Guar gum has long been investigated as a bread improver and anti-staling hydrocolloid (Guarda et al., 2004), while recent studies continue to show that formulation additives can influence moisture retention, texture and microbial stability during storage (Singh et al., 2024). Ascorbic acid is primarily used in breadmaking as an oxidizing improver through its role in strengthening the gluten network after conversion to dehydroascorbic acid. The microbiological stability of bread is affected by initial contamination, post-baking handling, packaging, storage temperature, water availability and the presence of preservatives. Moulds are common spoilage organisms in bakery products, while heat-resistant *Bacillus* spores can survive baking and contribute to rope spoilage under favourable storage conditions (Rosenkvist & Hansen, 1995; Samapundo et al., 2010). However, this study evaluated the effects of guar gum, ascorbic acid, Panok® and STK Royal on bacterial, coliform and mould counts of fresh wheat-cassava composite bread; crumb moisture retention and freshness during ambient storage; and presumptive microbial genera associated with the bread samples.

2. Materials and Methods

2.1. Materials

Commercial composite flour (Valeubra), granulated sugar, iodized salt, baker's yeast, and bread improvers (ascorbic acid, guar gum, Panok(R), and STK Royal) were used but procured from Gboko Main Market, Gboko Township, Benue State, Nigeria. The wheat-cassava composite flour includes 90% wheat flour and 10% cassava flour, consistent with the formulation used in bread-quality study (Ukoro et al., 2023). All ingredients were stored under refrigeration (approximately 10⁰ C) before use to maintain quality and prevent premature microbial contamination.

2.2. Bread formulation and preparation

Bread samples were prepared using a modified straight-dough method adapted from Akubor and Obiegbuna (2014). Formulation. The selection of dough improvers and optimization of processing conditions were based on our previous study carried out by Ukoro et al. (2023) on the Physical and sensory properties of breads prepared from wheat and cassava flour blend incorporated with different bread improver. The formulation consisted of wheat flour (59.10 g), sugar (10.31 g), salt (0.80 g), shortening (0.59 g), water (33.6 mL), baker's yeast (1.42 g), and calcium propionate (0.25 g). The bread improvers were incorporated into the wheat flour at concentrations of 0.5, 1.0, 2.0, and 3.0% (w/w). A control sample was prepared without any added improver. Calcium propionate was included in the base formulation for all treatments, including the control, and is therefore a common preservative background rather than an experimental treatment factor.

Dry ingredients were blended until homogeneous mixture was attained. Baker's yeast was activated in warm water (approximately 35 °C), added to the flour mixture and mixed to form dough. The dough was covered and fermented for 90 min at room temperature, kneaded to a smooth consistency, divided into approximately 150 g portions, moulded and proofed for 60 min at approximately 80% relative humidity. Loaves were baked in a preheated oven at 220 °C for 10 min, cooled to room temperature, packaged in low-density polyethylene bags and stored at 28 ± 2 °C until analysis.

Table 1: Recipe for the preparation of Wheat-Cassava composite bread

Ingredient	Amount in base formulation	Remarks
Composite flour	59.10 g	90% wheat flour : 10% cassava flour
Water	33.63 Ml	Added during mixing
Sugar	10.31 g	Granulated sugar
Yeast	1.42 g	Activated before mixing
Salt	0.80 g	Iodized salt
Shortening	0.59 g	Bakery shortening

Calcium propionate	0.25 g	Included in all treatments and control
Test improver	0.5, 1.0, 2.0 or 3.0% of composite flour	0% = control

Source: Hussan (2006). The improvers were incorporated into the wheat flour at 0.5, 1, 2, and 3%, respectively.

2.3 Storage stability and freshness studies

The fresh breads were stored in low polythene bags at room temperature for 4 days. Freshness index was expressed as a percentage of a crumb moisture value maintained at each storage time.

$$\text{Freshness index (\%)} = (\text{Moisture at storage day} / \text{Initial moisture}) \times 100$$

The samples were visually examined for any sign of fungal infestation, browning and other signs of deterioration.

2.4 Microbiological analysis

Ten grams of bread was aseptically dispersed in sterile 0.1% peptone water to obtain an initial 10^{-1} suspension, followed by serial decimal dilutions. Appropriate aliquots were plated on nutrient agar for total viable bacterial counts, MacConkey agar for coliform screening and potato dextrose agar supplemented with chloramphenicol for mould enumeration. Bacterial and coliform plates were incubated at 37 °C for 48–72 h and fungal plates at 25 °C for 48–72 h. Counts were expressed as colony-forming units per gram (CFU/g). Microbiological analyses were performed in triplicate using culture-based enumeration.

2.5 Isolation and identification of microorganisms

The pure cultures of bacteria were obtained by sub-culturing the colony on tryptone soy agar (TSA) plates by the streaking method before Gram staining and biochemical tests for bacterial identification. Staining was performed using standard procedure. Colonial morphology, microscopic examination at x40 magnification and biochemical tests were used for the preliminary identification of the fungal isolates following the procedure of Anibijuwon and Sunday (2012).

2.6. Experimental design and statistical analysis

The experiment followed a completely randomized 4×4 factorial design comprising four bread improvers such as guar gum, ascorbic acid, Panok® and STK Royal, each applied at 0.5, 1.0, 2.0 and 3.0% (w/w), with an untreated control. Data from treated samples were analysed using two-way ANOVA to assess the effects of improver type, concentration and their interaction, while storage data were evaluated with an appropriate repeated-measures or mixed-effects approach. Microbial counts were expressed as CFU/g and log₁₀-transformed where necessary to satisfy statistical assumptions. Results were considered significant at $p < 0.05$.

3. Results

3.1 Microbial quality of fresh bread

The microbial profiles of the fresh bread samples differed remarkably among improver treatments and concentrations (Table 2). Guar-gum breads had the highest bacterial counts among the treated samples, ranging from 2.33×10^4 to 2.88×10^4 CFU/g. Ascorbic-acid breads had the lowest bacterial counts, ranging from 2.47×10^2 to 2.87×10^2 CFU/g. STK Royal samples also showed relatively low bacterial counts (3.34×10^2 to 3.37×10^2 CFU/g), whereas Panok® samples shows approximately 2.07×10^4 CFU/g across the concentrations reported. The control bacterial count was 2.67×10^3 CFU/g.

The fungal pattern was different from the bacterial pattern. Guar-gum breads had the lowest mould counts (2.37×10^2 to 2.67×10^2 CFU/g), followed by ascorbic-acid breads (3.23×10^2 to 3.32×10^2 CFU/g) and STK Royal breads (3.43×10^2 to 3.83×10^2 CFU/g). Panok® breads had higher mould counts (1.13×10^3 to 1.43×10^3 CFU/g), although these values remained below the control mould count of 5.83×10^3 CFU/g. Coliform growth was not detected in the fresh samples.

Table 2. Microbial counts of fresh wheat-cassava composite bread containing different bread improvers

Improver	Level (%)	Total bacterial count (CFU/g)	Total mould count (CFU/g)	Coliform
Guar gum	0.5	2.33×10^4	2.67×10^2	ND
Guar gum	1.0	2.63×10^4	2.37×10^2	ND

Guar gum	2.0	2.83×10^4	2.57×10^2	ND
Guar gum	3.0	2.88×10^4	2.59×10^2	ND
Ascorbic acid	0.5	2.67×10^2	3.23×10^2	ND
Ascorbic acid	1.0	2.87×10^2	3.32×10^2	ND
Ascorbic acid	2.0	2.47×10^2	3.32×10^2	ND
Ascorbic acid	3.0	2.87×10^2	3.23×10^2	ND
Panok®	0.5	2.07×10^4	1.43×10^3	ND
Panok®	1.0	2.07×10^4	1.23×10^3	ND
Panok®	2.0	2.07×10^4	1.13×10^3	ND
Panok®	3.0	2.07×10^4	1.33×10^3	ND
STK Royal	0.5	3.37×10^2	3.83×10^2	ND
STK Royal	1.0	3.36×10^2	3.43×10^2	ND
STK Royal	2.0	3.35×10^2	3.51×10^2	ND
STK Royal	3.0	3.34×10^2	3.53×10^2	ND
Control	0	2.67×10^3	5.83×10^3	ND

Values are expressed as colony-forming units per gram (CFU/g). ND = not detected. Guar gum, ascorbic acid, Panok® and STK Royal were incorporated at 0.5, 1.0, 2.0 and 3.0% (w/w), while the control contained no added improver.

3.2 Crumb moisture and freshness during storage

Crumb moisture reduces during storage in all samples (Table 3A). STK Royal retained the greatest moisture by day 4 (11.75%), while the control retained the least (8.51%). Guar gum, ascorbic acid and Panok® recorded day-4 moisture values of 9.80%, 10.68% and 9.96%, respectively. Because the starting moisture values differed among treatments, the freshness index provides a relative measure of moisture retention from each sample's own baseline.

Table 3A. Crumb moisture (%) during four days of ambient storage

Improver	Day 0	Day 1	Day 2	Day 3	Day 4
Guar gum	14.00	13.84	11.87	11.48	9.80
Ascorbic acid	17.30	14.53	12.98	12.19	10.68
Panok®	16.00	13.88	12.48	10.80	9.96
STK Royal	16.10	14.93	13.32	12.59	11.75
Control	14.00	11.59	11.09	10.26	8.51

The values represent the measured parameter for samples treated with different improvers during storage from Day 0 to Day 4.

The freshness index also reduces progressively during storage (Table 3B). STK Royal had the highest Day-4 freshness index at 73.00%, followed by guar gum at 70.00%. Panok® and ascorbic acid had 62.25% and 61.75%, respectively, whereas the control showed the lowest retention at 60.75%. Thus, STK Royal showed the greatest proportional retention of the measured freshness parameter over the four-day storage period.

Table 3B. Freshness index (%) during four days of ambient storage

Improver	Day 0	Day 1	Day 2	Day 3	Day 4
Guar gum	100.00	98.75	84.75	82.00	70.00
Ascorbic acid	100.00	84.00	75.00	70.50	61.75
Panok®	100.00	86.75	78.00	67.50	62.25
STK Royal	100.00	92.75	82.75	78.25	73.00
Control	100.00	82.75	79.25	73.25	60.75

The values represent percentage retention of the measured freshness parameter relative to the initial Day-0 value of each treatment.

3.3 Presumptive spoilage organisms

The culture morphology, microscopic characteristics, Gram reaction and biochemical observations supported the presumptive identification of bacterial isolates which belongs to the genera *Bacillus*, *Micrococcus* and *Staphylococcus*. Fungal isolates were presumptively identified belonging to the genera *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor*. These organisms were reported at the genus level of classification because identification was based on conventional physical characteristics.

4. Discussion

4.1 Microbial responses to bread improver

The microbial report showed that the effects of the bread improvers differed between bacterial and fungal populations. Ascorbic acid recorded the lowest bacterial counts, whereas guar gum recorded the lowest mould counts. More so, bacterial counts in the guar gum formulations exceeded that of the control, despite their comparatively low mould counts. These contrasting responses indicate that no single improver provided the most favourable outcome for both microbial groups. Rather, each formulation exhibited a distinct microbial report. The relatively low bacterial counts observed in the ascorbic acid formulations should be considered in relation to its established technological function in breadmaking. Ascorbic acid is primarily used as a dough-strengthening agent and undergoes oxidation to dehydro-ascorbic acid during dough development, thereby participating in reactions that influence dough structure and breadmaking performance (Beghin et al., 2025). Since pH, water activity and redox potential were not measured, and direct antimicrobial activity was not evaluated, the lower bacterial counts associated with ascorbic acid cannot be attributed to a specific antibacterial mechanism. The findings instead indicate an association between the ascorbic acid treatment and reduced bacterial recovery under the conditions of the study.

Guar gum showed a markedly different microbial pattern. It produced the lowest mould counts but the highest bacterial counts among the treated breads. Hydrocolloids influence water binding, dough rheology, crumb structure and moisture distribution, and their technological effects depend on their physico-chemical properties, concentration and interactions with other constituents of the food matrix (Guarda et al., 2004; Parsamajd et al., 2025). The contrasting bacterial and mould counts observed with guar gum therefore suggest that modification of the bread matrix may have influenced the two microbial groups differently. The effect of concentration was also dependent on the improver. Bacterial counts in guar gum breads increased from 2.33×10^4 CFU/g at 0.5% to 2.88×10^4 CFU/g at 3.0%, whereas Panok® maintained the same bacterial count of 2.07×10^4 CFU/g across all concentrations. STK Royal also showed only a narrow range in bacterial counts. Mould counts did not exhibit a consistent directional response to increasing concentration. Therefore, the descriptive results do not indicate a general dose-dependent microbial effect applicable to all four improvers.

4.2 Moisture retention and staling behaviour

The decrease in crumb moisture and freshness during storage is consistent with bread staling, which involves moisture redistribution and structural changes in starch, particularly amylopectin retrogradation. Recent bread research continues to show close relationships among water redistribution, crumb firming and staling kinetics during storage (Pyo et al., 2024). The present data show that STK Royal retained the highest day-4 moisture and freshness index, while the control showed the lowest values.

The superior freshness retention of STK Royal may reflect the combined technological action of components within the commercial formulation, but its proprietary composition was not reported. It is therefore inappropriate to assign the effect specifically to an enzyme, emulsifier or hydrocolloid without product-composition evidence. Guar gum also retained freshness better than the control, which is consistent with the established ability of hydrocolloids to modify water binding and delay crumb firming in bread systems (Guarda et al., 2004). Recent formulation studies similarly demonstrate that additives capable of altering the bread matrix can improve moisture retention and storage quality, although effects are highly formulation-dependent (Singh et al., 2024).

Ascorbic acid and Panok® provided much smaller day-4 freshness advantages over the control than STK Royal and guar gum. This reinforces the central finding that microbial control and anti-staling performance are separate functional outcomes. An improver associated with low microbial counts is not necessarily the improver that best preserves crumb freshness.

4.3 Presumptive spoilage organisms and implications for bread quality

The presumptive bacterial isolates comprised *Bacillus*, *Micrococcus* and *Staphylococcus*, whereas the fungal isolates included *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor*. The recovery of these organisms is consistent with the diverse microbiota associated with cereal raw materials, processing environments and bakery products. Recent assessments of bread microbiological quality identify *Aspergillus*, *Penicillium* and other filamentous fungi among organisms associated with mould spoilage of bakery products. The presumptive occurrence of *Bacillus* is relevant to bread quality because some members of this genus are associated with rope spoilage. Their endospores can display sufficient heat resistance to survive baking and may subsequently proliferate under favourable storage conditions, producing characteristic deterioration of the crumb (Pacher et al., 2022). However, the isolates in the present study were identified only presumptively, and the results therefore indicate the occurrence of *Bacillus*-like isolates rather than confirmed rope-spoilage species.

Similarly, the recovery of *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* is principally relevant to the spoilage potential of the bread. *Aspergillus*, *Penicillium* and *Rhizopus* are among the fungal genera frequently associated with bread deterioration. The present findings establish presumptive fungal occurrence but do not establish species identity or production of fungal toxins. The absence of detectable coliforms across all fresh bread samples provides an additional indication of the microbiological condition of the products at the time of analysis. Nevertheless, the finding is appropriately reported as “not detected” because analytical non-detection does not establish absolute absence below the detection capability of the method.

4.4 Recommendations

Further studies incorporating microbial enumeration throughout storage, water activity, pH, instrumental texture analysis, sensory evaluation and confirmatory microbial identification would provide a more comprehensive assessment of storage stability and facilitate optimisation of improver formulations for wheat-cassava composite bread.

5. Conclusion

Commercial bread improvers produced distinct effects on the microbial quality and moisture-related freshness characteristics of wheat-cassava composite bread. Ascorbic acid was associated with the lowest bacterial counts, while guar gum recorded the lowest mould counts among the treated samples. STK Royal provided the greatest retention of crumb moisture and freshness during four days of ambient storage. No single improver therefore provided the most favourable response across all measured quality attributes. The findings demonstrate that microbial quality and moisture-related freshness represent distinct formulation responses. The comparatively low bacterial counts associated with ascorbic acid did not correspond to the highest freshness retention, while guar gum combined low mould counts with comparatively high bacterial counts. STK Royal, in contrast, exhibited the strongest moisture-retention performance. These treatment-specific responses highlight the need to select bread improvers according to the particular quality attribute targeted in wheat-cassava composite bread production.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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